Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

A deeper insight to an intriguing acetonitrile-water binary mixture: synergistic effect, dynamics stokes shift, fluorescence correlation spectroscopy and NMR studies.

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1. Experimental section:

1.1 Materials and method: *Coumarin 343* (*C343*), *4-(dicyanomethylene)-2-methyl-6-(4-dimethylaminostyryl)-4H-pyran* (*DCM*), *Rhodamine-6G* (*R6G*), *acetonitrile* and *isopropanol* were purchased from Sigma-Aldrich and used without further purification. *4-aminophthalimide* (*4-AP*) was received form Kodak and recrystallized from *methanol-water* mixture. *Hydrochloric acid* and *sodium hydroxide* were purchased from *Spectrochem* and were used for the preparation of acidic and basic solutions of *ACN-WT* mixture for the *TRANES* study. *NMR* solvents, *acetonitrile-d3* and *deuterium oxide* (D_2O) were purchased from *Acros Organics*. All the experiments were carried out in room temperature (*298K*). The dye solutions were allowed to stabilize for several hours to ensure complete dissolution prior to any optical measurement.

1.2 Instruments used: Steady state absorption and emission spectra were recorded by using *Perkin-Elmer Lambda-750* spectrophotometer and *Perkin-Elmer LS55* spectrofluorometer, respectively. We used 0.5 nm/0.5 nm (excitation /emission) slits to obtain highly accurate emission peak positions from the dye molecules. Similar low slit widths were used for absorption spectra measurements. We took average of absorption peak positions obtained from four sets of measurements for a same sample (same dye within a same composition of binary mixture). These four sets of same samples were prepared separately. We observed absorption peaks (and emission peaks) from all these four sets appear within ±0.5 nm range from the average absorption peak (emission peak) position. Fluorescence lifetimes were measured by using time correlated single photon counting (TCSPC) setup (Edinburgh, OB920) with an instrumental response function of ~70 ps. Lifetimes were fitted by reconvoluting with excitation lamp profile. Excitation lamp profile was collected using the scattering from Ludox solution. All lifetime measurements were performed at magic angle.

^{1}*H NMR* spectra were recorded in Bruker AV-400 spectrometer. The chemical shift data reported are downfielded with respect to tetramethyl silane (TMS).

<u>Fluorescence correlation spectroscopy (FCS) measurement:</u>

FCS studies were carried out by using a confocal microscope (*Zeiss LSM780*). Pinhole diameter was kept fixed at 40 μm and excitation wavelengths were chosen 458 nm for *DCM* and 488 nm for *R6G*, respectively. Calibration of the excitation volume was done by using a sample (*R6G* in water) whose diffusion coefficient is reported (D_t=*426μm^2/sec*).[1] Transverse

radius (ω_{xy}) of the excitation volume was calculated using the relation, $\omega_{xy}=\sqrt{(4\tau_D D_t)}$; where D_t is known (*~426μm²/sec* for R6G in WT) and τ_D (diffusion time) was obtained from the fitting of autocorrelation curve (of *R6G* in *WT*) using equation *S1*. We obtained ω_{xy}~278 nm for our setup. The effective excitation volume was determined using the equation $V_{eff} = \pi^{3/2} k(\omega_{xy})^3$; where k [k=($\omega_z/\omega_{xy}$)=5] is the structure parameter and ω_z is the axial radius of the excitation volume.[2] The calculated value of the excitation volume was found to be ~0.6 femto litre. We fitted the normalized auto-correlation curves of dye molecules in *ACN-WT* mixture by using the following equation (equation S1).[1-2]

$$G(\tau) = (1 - T + T\exp^{-\tau/\tau_{triplet}})\left(\frac{1}{1+\tau/\tau_D}\right)\left(\frac{1}{1+(\tau/\mathrm{k}^2\tau_D)}\right)^{1/2} \quad \text{(S1)}$$

Where, T is the fraction of molecules in triplet state and $\tau_{triplet}$ is the corresponding lifetime in triplet state. τ_D is the diffusion time of the fluorescent dye molecule along the transverse direction. We obtained numerical value of τ_D from the fitting of autocorrelation curve. Using the known values of τ_D and ω_{xy}, one can easily get the value of translational diffusion coefficient (D_t) of the fluorescent dye molecule using the following equation.

$$\tau_D = \frac{\omega_{XY}^2}{4D_t} \quad \text{(S2)}$$

References

1. Z. Petrasek and P. Schwille, *Biophys. J.*, 2008, **94**, 1437-1448.
2. S. Ghosh, U. Mandal and A. Adhikari, *Chem. Asian. J.*, 2009, **4**, 948-954.

Figure S1. Plot of *Onsager polarity function* $F(\varepsilon)[= 2(\varepsilon-1)/2\varepsilon+1]$ as function of mole fraction of water in the *ACN-WT* binary mixture.

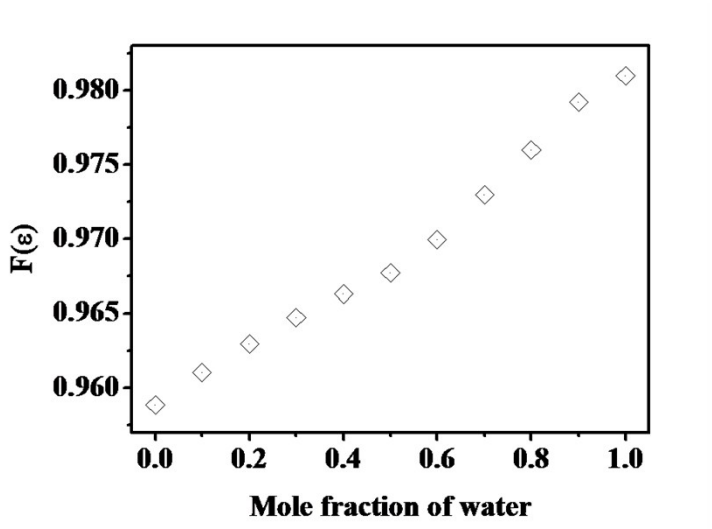
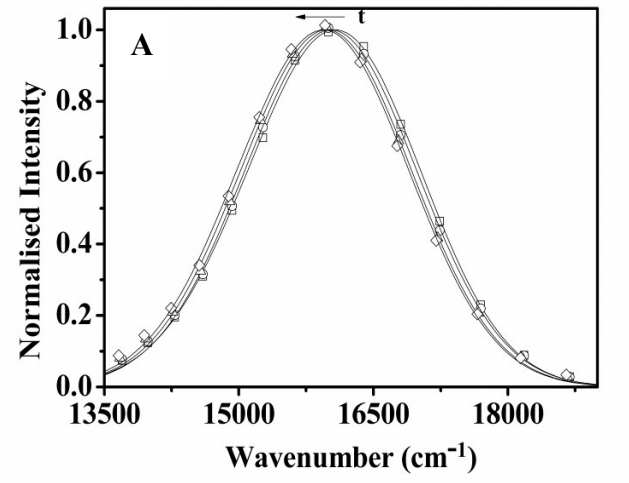
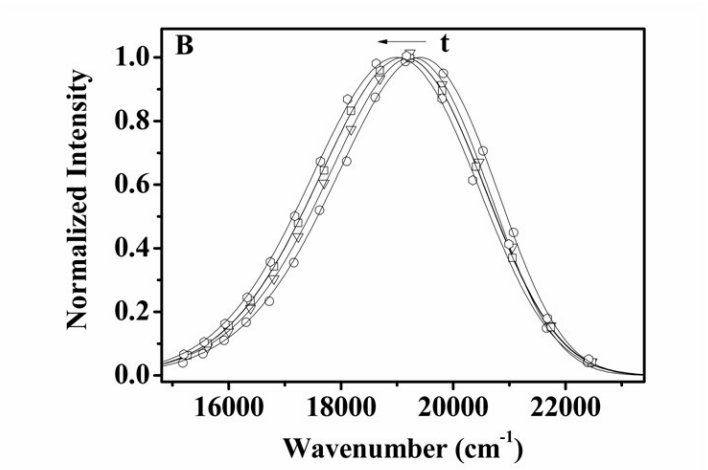


Figure S2. Time resolved emission spectra (*TRES*) of (A) *DCM*, (B) *4-AP* and (C) *C343* in *ACN-WT* binary mixture (X_{WT}=0.51) at time 0 ps, 200 ps, 500 ps, 1200 ps, respectively.

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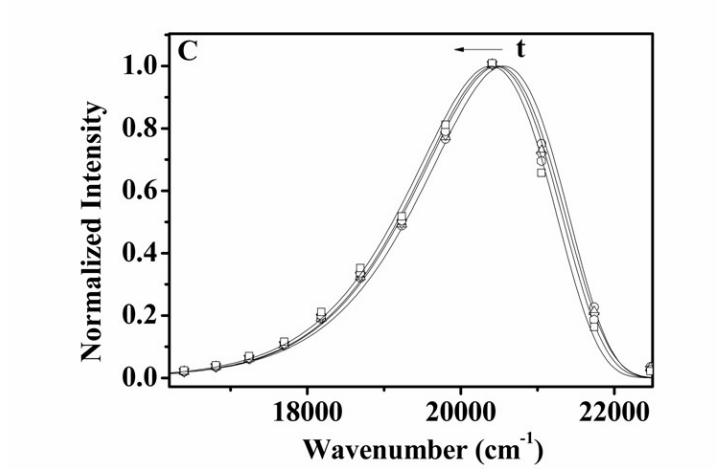
Wavenumber (cm-1)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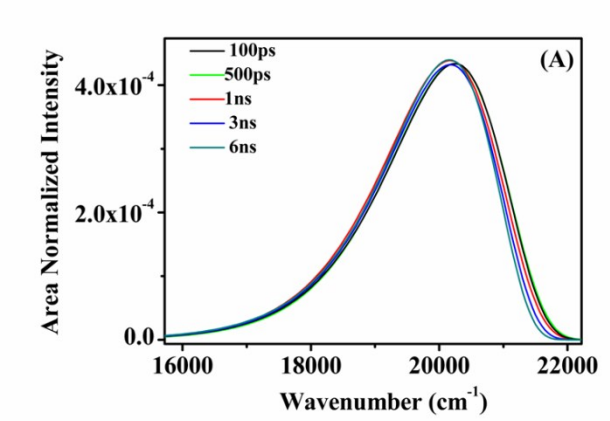
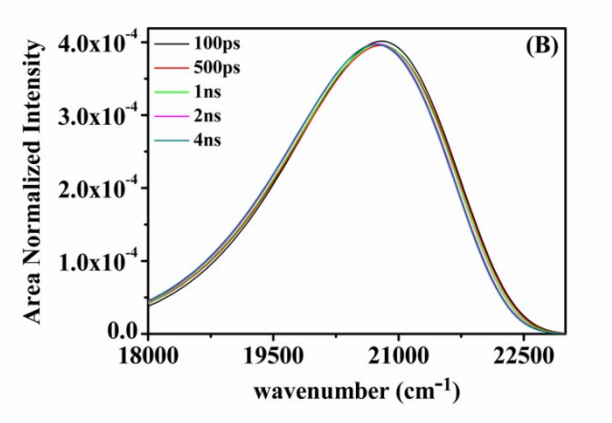
Wavenumber (cm-1)

Figure S3. *TRANES* of *C343* in (A) acidic (*1 N HCl*) *ACN-WT* binary mixture, (B) basic (*5mM NaOH*) *ACN-WT* binary mixture and (C) in isopropanol. No isoemissive point is detected in any of the following cases.





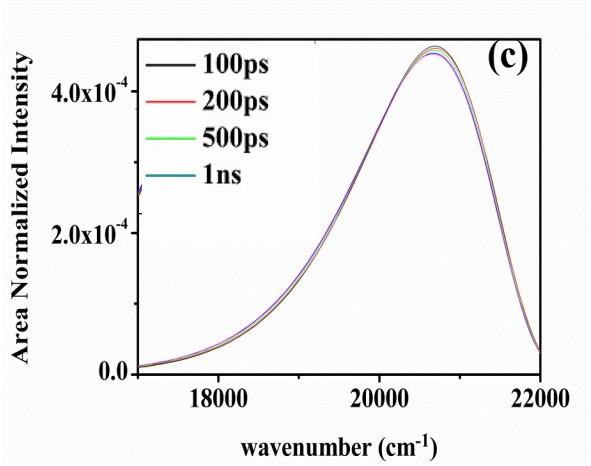
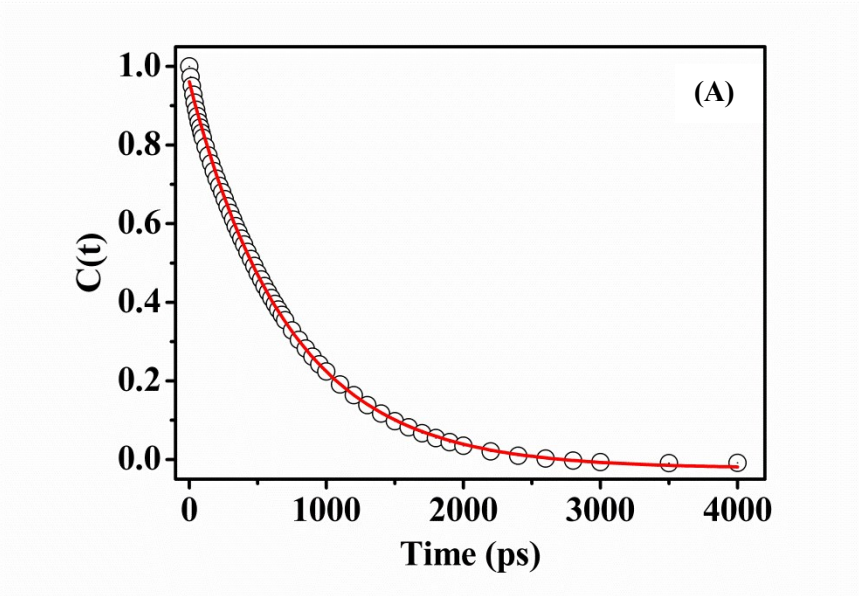


Figure S4. Decay of solvent correlation function for *C343* in *ACN-WT* binary mixture in (A) basic condition (*5 mM NaOH*) and (B) Acidic condition (*1N HCl*).



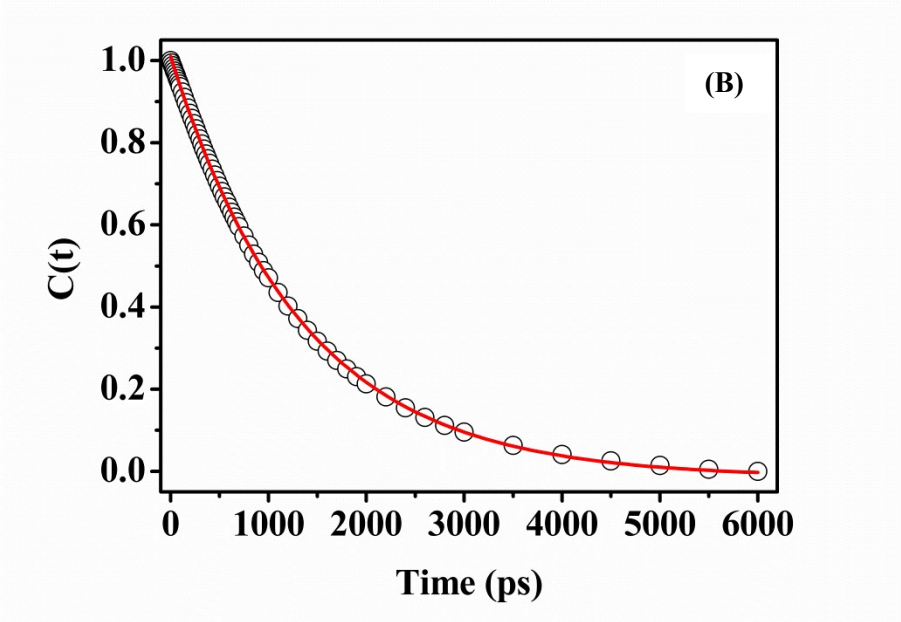


Figure S5. (A) Autocorrelation curve of *R6G* with fitted line (red line) in *ACN-WT* binary (X_{WT} = 0.51) mixture for a particular *X-Y-Z* position inside the sample. (B) Few fitting curves of autocorrelation functions of *R6G* in *ACN-WT* (X_{WT} = 0.51) binary mixture as obtained from different X-Y-Z positions inside the sample.

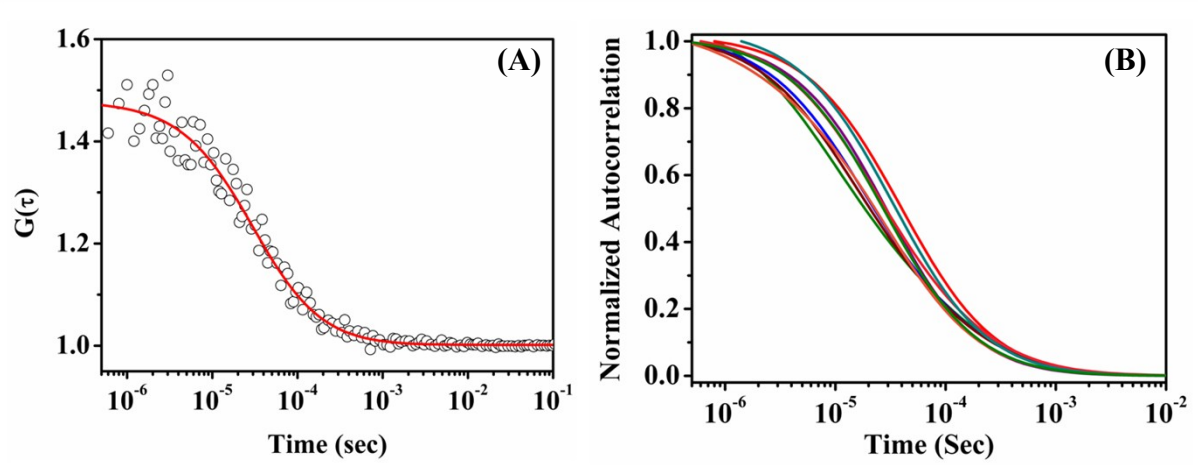
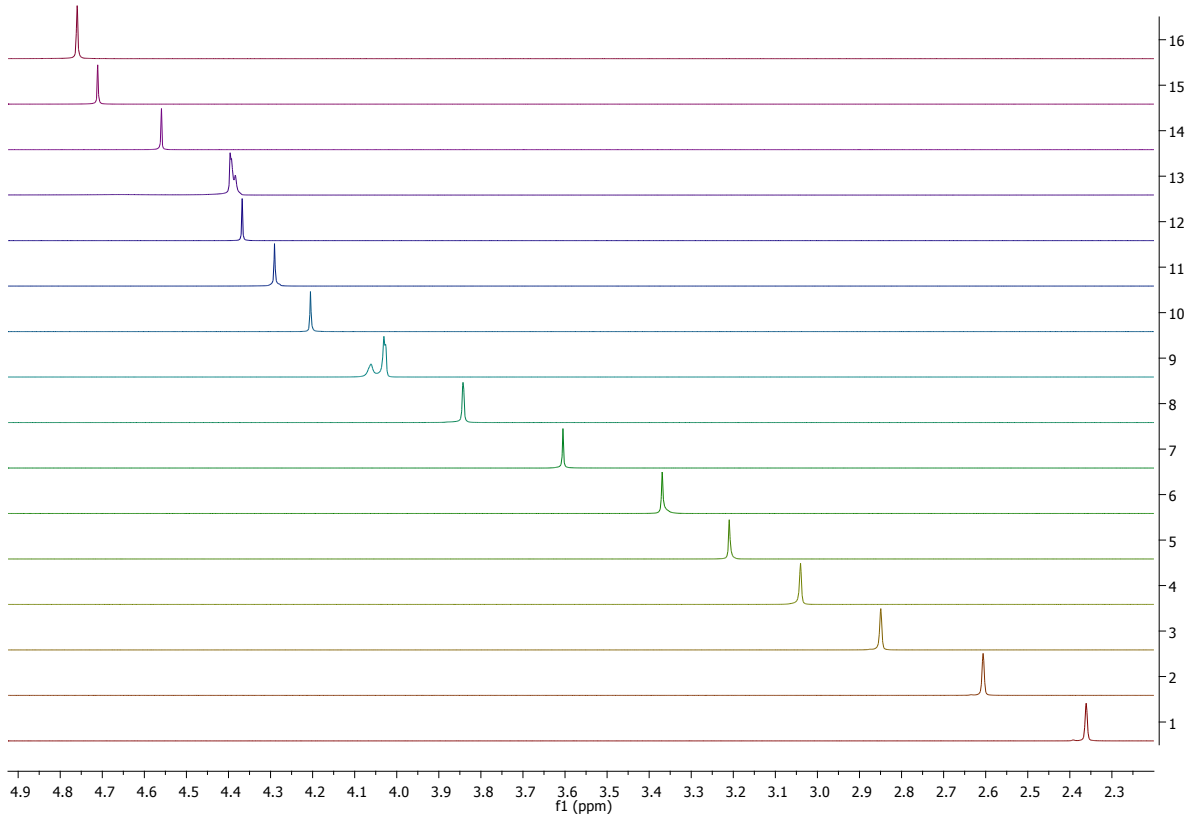
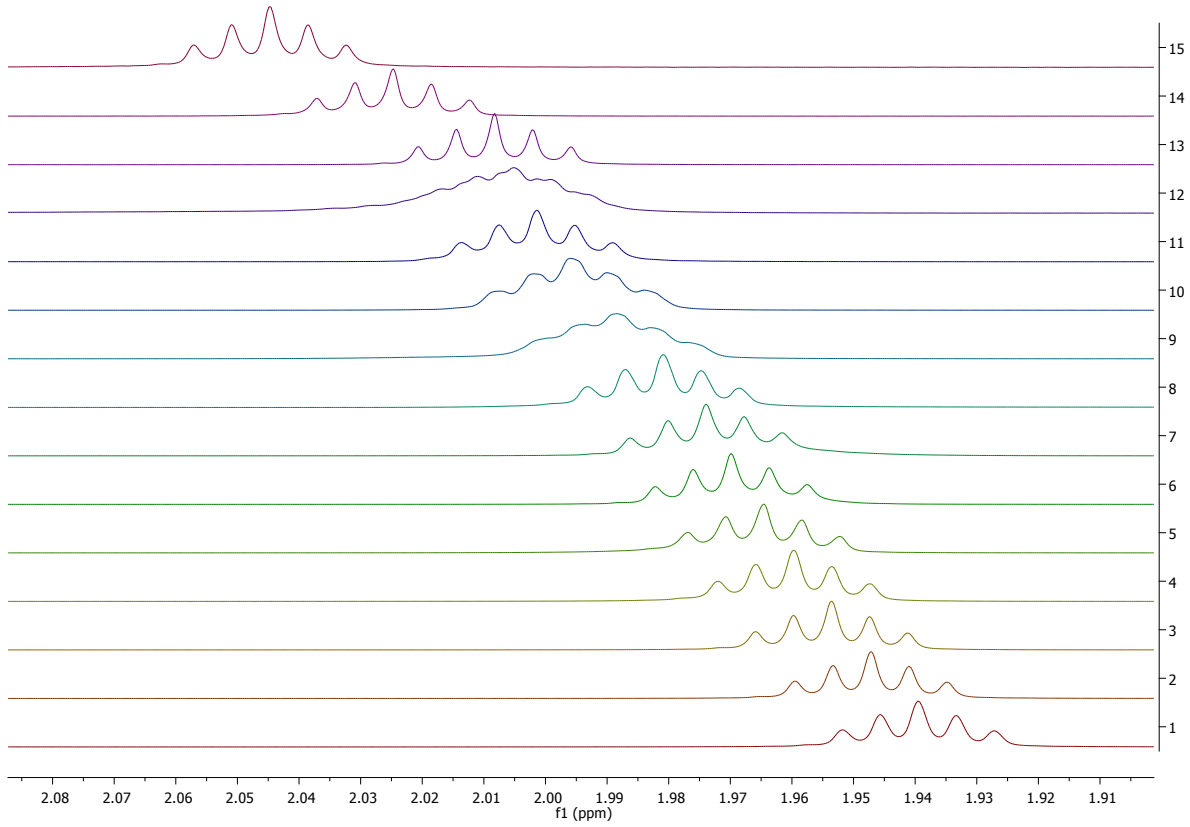


Figure S6. (A) Chemical shift data for the *O-H* proton of water and (B) Chemical shift data for the *C-H* proton of acetonitrile in the binary mixture at different compositions.

(A)



(B)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